Rapid development of microsatellite markers for *Culicoides obsoletus* using Next Generation Sequencing

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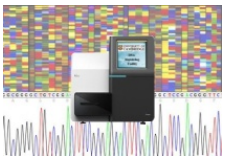

- **Importance of Palearctic Culicoides species as vector in the emergence of bluetongue and Schmallenberg viruses in northern Europe**. *Culicoides obsoletus* is an abundant, largely distributed suspected vector species of these two *Culicoides*-borne diseases.
- Need to characterize *Culicoides* dispersal to investigate fine-scale and regional spread patterns and assess genetic structure over the geographical distribution. **Interest for practical recommendations on vector control and restrictions movements.**
- Population genetics approach is powerful to provide information on insect movement at spatial and temporal scales. It is a good alternative to mark-release-recapture method not-well suited for assessing long range dispersal and logistically very demanding.

⇒ **Objective and strategy: development of microsatellite markers for *C. obsoletus* using Next Generation Sequencing (NGS).**

C. obsoletus
DNA samples from
several geographic localities
France, Greece, UK

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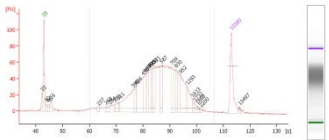
Paired-end DNA library
(Illumina Nextera® technology)



Quality control

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illumina®

Paired-end sequencing
(MiSeq sequencer)

2 299 075 reads
(2 x 300 bp)

Bioinformatic pipeline

de novo assembly
(ABySS software)

824 373 contigs

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SSR loci search
di- and tri- nucleotide motifs
(MISA tool)

82 276 SSR loci detected

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Primer design
(Primer3 software)

43 676 primer pairs

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SSR selection
Dinucleotide motifs
Exclusion of compound SSR
More than 12 repeat units
Tm around 55°C
Amplification size: 150-350 bp

214 SSRs

Optimization of PCR conditions

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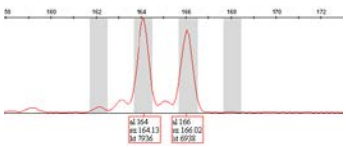
First validation of polymorphism
(ABI 3500 genetic analyzer)
8 individuals within the species geographic range

11 loci



	SSR	PRODUCT size (Bp)	size range (Bp)
obsMS1	(CA)19	152	126-195
obsMS2	(TG)14	160	140-182
obsMS3	(CA)13	161	149-163
obsMS4	(GA)12	175	162-176
obsMS5	(TG)15	175	149-193
obsMS6	(TA)12	187	175-201
obsMS9	(CA)12	240	229-299
obsMS10	(AC)17	257	227-257
obsMS11	(CT)12	283	276-289
obsMS12	(CA)14	340	320-335
obsMS13	(CA)12	266	252-281

↓

multiplex PCR conditions

- **NGS approach: a rapid and relatively inexpensive way of yielding hundreds of potential markers.**
- These markers will provide a good tool for population genetics analysis of *C. obsoletus.* **The next steps are to test the loci polymorphism and deviation from Hardy Weinberg equilibrium as well as the presence of null alleles in a larger sample.**
- The perspectives are to explore the influence of landscape features on *C. obsoletus* dispersion at the local scale, and to investigate at regional scale whether long-distance wind-borne dispersal shape the population structure.
- **Cross-amplification was also observed with *C. scoticus*,** a sympatric species of *C. obsoletus* widely distributed in the Palearctic region. This will also allow to study the influence of the biology of these species on their dispersal patterns.

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